## REMARKS

Claims 29, 30, 32-39, 41, 44, 46-52, and 59-63 are pending with claims 44, 62, and 63 allowed. Applicant has amended claims 29, 47, 48, and 59. No claims have been cancelled or withdrawn in this paper.

**Claim Amendments**

Applicant amended independent claims 29, 47, 48, and 59 to more clearly claim different aspects of the invention. Support for these amendments may be found throughout the specification.

Applicant amended claim 29 to recite:

a) treating the biological sample with a reagent comprising phenol that when added to the biological sample a final phenol concentration is from about 10%$^{w/w}$ to about 60%$^{w/w}$ and at least one ribonuclease inhibitor,

b) mixing the mixture containing biological sample from step (a) with at least one hydrophobic solvent and a buffer at a concentration sufficient to maintain a pH in the range from about pH 3.6 to below pH 4.0,

c) separating an aqueous phases from the mixture obtained in step (b) by sedimentation and recovering purified RNA from an aqueous phase by precipitation with about an equal volume of a water-soluble organic solvent which is RNA that does not reveal the presence of DNA when assayed by reverse transcription polymerase chain reaction (RT-PCR), and

d) washing and solubilizing the precipitated RNA.

Applicant similarly amended claim 47 to recite:

a) treating the biological sample with a mono-phase reagent comprising phenol at a final concentration ranging from about 3%$^{w/w}$ to less than 30%$^{w/w}$ and a buffer sufficient to maintain a pH of the resulting mixture containing biological sample in the range from about pH 3.6 to about pH 5.5,

b) sedimenting or filtering the mixture containing biological sample to obtain a purified biological sample substantially free of DNA, proteins, and cellular components without the use of a hydrophobic solvent and performing phase separation,

c) adding to the purified biological sample about an equal volume of a water-soluble organic solvent to precipitate purified RNA which is RNA that does not reveal the presence of DNA when assayed by reverse transcription polymerase chain reaction (RT-PCR),

d) sedimenting or filtering the precipitated RNA,and

e) washing and solubilizing the precipitated RNA.

Applicant similarly amended claim 48 to recite:

a) treating the biological sample with a mono-phase reagent comprising phenol at a final concentration ranging from about 3%$^{w/w}$ to less than 30%$^{w/w}$, at least one chaotrope, and a buffer sufficient to maintain a pH of the resulting mixture containing biological sample in the range from about pH 3.6 to about pH 5.5,

b) sedimenting or filtering the mixture containing biological sample to obtain a purified biological sample substantially free of DNA, proteins, and cellular components,

c) adding to the purified biological sample at least one hydrophobic organic solvent and a buffer in a concentration sufficient to maintain a pH of the purified biological sample in the range from about pH 3.6 to below pH 4.0,

d) recovering purified RNA from an aqueous phase to which about an equal volume of a water soluble organic solvent is added to precipitate purified RNA which is RNA that does not reveal the presence of DNA when assayed by reverse transcription polymerase chain reaction (RT-PCR),

e) sedimenting or filtrating the precipitated RNA, and

f) washing and solubilizing the precipitated RNA.

Applicant deleted the phrase "without performing phase separation" from step (b) of claim 48. A version of this element was first added to the claim in an Amendment and Request for Continued Examination on April 20, 2007. Applicant believes that this element is no longer necessary to distinguish this claim from the cited art.

Applicant amended claim 59 to recite:

treating the biological sample with an aqueous composition comprising phenol at a final concentration ranging from about 1%$^{w/w}$ to about 60%$^{w/w}$, at least one chaotrope, a buffer in a concentration sufficient to maintain a pH of the

> composition in the range from about pH 2.0 to about pH 9.0, at least one water-soluble organic solvent at a concentration from about 10%$^{w/w}$ to about 40%$^{w/w}$ to selectively precipitate higher molecular weight RNA from the biological sample, and
>
> precipitating purified higher molecular weight RNA from the biological sample.

**Rejections under 35 U.S.C. § 112**

Examiner rejects claims 59-61 under 35 U.S.C. § 112, second paragraph, as allegedly indefininte. Applicant respectfully disagrees. Applicant's specification clearly states "[o]rganic solvents at concentrations from about 10% $^{w/w}$ to about 40% $^{w/w}$ precipitate RNA molecules greater than about 200 bases, considered as higher molecular weight RNA." Applicant's paragraph [0053] (emphasis added). Thus, Applicant submits that the specification provides a clear standard for determining the scope of the claim and respectfully requests that the rejection be withdrawn.

**Rejections under 35 U.S.C. § 103**

Examiner rejects claims 29, 30, 32-39, 41, 46, 52, and 59-61 under 35 U.S.C. § 103(a) as allegedly unpatentable over Chinese Patent 1,220,995 to Chen ("Chen") in view of U.S. Patent No. 5,346,994 to Chomczynski ("Chomczynski"). Applicant respectfully disagrees.

Regarding claims 29 and 59, Examiner states that:

> It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention was made to modify the isolation buffer of Chen, who notes a desire to 'regulate pH value (see page 3)', to incorporate enough buffering component as taught by Chomczynksi since Chomczynski notes 'the solvent solution may include a buffering component, such as sodium acetate or sodium citrate, in an amount sufficient to maintain the pH of the solution (see column 3, lines 17-22).' An ordinary practitioner would have been motivated to include sufficient buffering in the isolation buffer of Chen in order to maintain the pH since both Chen and Chomczynski teach and motivate the use of buffering components to maintain the pH of the solution.

Office Action, page 6. Applicant respectfully submits, that even if Examiner's characterization of the teachings and motivations in Chen and Chomczynski are correct, a point Applicant does not concede, the combination of these references still fails to render obvious the present claims. Applicant's claim 29 requires more than merely buffering an extraction composition; Applicant's claim requires buffering the extraction composition to maintain the pH in a specified range from about pH 3.6 to below pH 4.0 during the extraction of RNA from a biological sample. Accordingly, Applicant has amended step (b) to recite "mixing the mixture containing biological sample from step (a) with at least one hydrophobic solvent and a buffer at a concentration sufficient to maintain a pH in the range from about pH 3.6 to below pH 4.0" (emphasis added). Again, neither Chen nor Chomzcynski, individually or in combination, teach or suggest maintaining the pH in a range from about 3.6 to below pH 4.0 during the extraction of RNA and therefore cannot provide any motivation to maintain the pH over this range.

Examiner also states that "a skilled artisan would have recognized that the addition of buffering component in an amount sufficient to maintain the pH of the isolation solution would have avoided unwanted radical pH change during isolation." Examiner's statement presupposes that prior to the present invention, the skilled artisan would have wanted to maintain the pH in the presently claimed range. Applicant again submits nothing in Chen or Chomczynski discloses or contemplates buffering the extraction composition to maintain the pH of the composition when mixed with the biological sample in the range from about pH 3.6 to below pH 4.0.

Examiner makes reference to Chen's mention of a "pH regulator" but, as fully discussed in responses to earlier Office Actions, the concentrations of the so-called pH regulator

are miniscule (i.e., 0.001%-0.005% for trisodium citrate and 0.005%-0.01% for sodium acetate). These miniscule amounts of pH regulator are insufficient to maintain the pH of a mixture of the composition and a biological sample in the range from about pH 3.6 to below pH 4.0. Again, Applicant points out that the pH of most biological samples have a pH above 7 and are themselves buffered to maintain this near neutral pH. Therefore, the very low concentrations of the pH regulator disclosed in Chen would provide virtually no buffering capacity, much less the buffering capacity to acidify a biological sample that is itself buffered to maintain a neutral or neutral pH. Thus, Chen fails to disclose or contemplate buffering sufficient to maintain the pH in the claimed range.

Examiner also states that "Chen teaches a method of isolating purified RNA from a biological sample." Office Action, page 3. Applicant respectfully submits that Chen does not contemplate isolating purified RNA from a biological sample as presently claimed. Applicant's claim 29 requires "RNA that does not reveal the presence of DNA when assayed by reverse transcription polymerase chain reaction (RT-PCR)." In contrast, Chen is concerned with quickly extracting "total RNA" and never discusses extracting purified RNA that meets this definition. In addition, Chen does not disclose or contemplate a composition or method that would yield purified RNA that meets this definition.

In Applicant's Amendment filed on January 17, 2007, Applicant submitted his declaration under 37 C.F.R. § 1.132 ("the Declaration") providing evidence directly contradicting Examiner's position that Chen results in the extraction of purified RNA. Examiner has repeatedly stated that the Declaration is not sufficient to show unexpected results of Applicant's claimed method relative to Chen. Applicant respectfully submits that the Declaration is not being referenced now to show the unexpected results of Applicant's claimed invention, but

instead to show that the Chen method is different Applicant's claimed method.[1] Specifically, Applicant's claim 29 requires, in part, that the method yield "RNA that does not reveal the presence of DNA when assayed by reverse transcription polymerase chain reaction (RT-PCR)." Chen discloses two compositions. In the Declaration, Applicant presented data collected from RT-PCR assays for DNA contamination conducted with RNA extracted using the Chen compositions and methods. Those data clearly demonstrate that the two Chen compositions yield RNA that is contaminated with DNA when assayed by RT-PCR. The very fact that Chen yields RNA that is contaminated with DNA to a level that is detectable by RT-PCR clearly indicates that the compositions and methods disclosed in Chen do not disclose or contemplate claim 29. Thus, Chen not only fails to contemplate compositions or methods that might result in purified RNA, the compositions and methods that are disclosed in Chen do not in fact yield purified RNA that is free of contamination by DNA as determined by RT-PCR and as required by claim 29.

Additional support for lack of purity of RNA extracted using the methods of Chen are found in Chen's own words. Chen's stated objective is the relatively quick and inexpensive extraction of high quantities of total RNA from biological samples, not highly pure RNA. *See*, for example, Chen, abstract, page 4, second paragraph, and page 7, last paragraph. Chen further states that the extracted must be reconstituted and stored in a "suitable amount of RNA protective agent." Chen, pages 3 and 7. If Chen yielded purified RNA, this protective agent would not be necessary.

[1] Thus, Examiner's position regarding the requirement that the evidence of unexpected results must be commensurate with the scope of the claims is not presently at issue.

Thus, Chen and Chomczynski, individually or in combination, clearly do not disclose or contemplate methods of purifying RNA from a biological sample by buffering the extraction composition so that the extraction is conducted in the range from about pH 3.6 to below pH 4.0. For at least these reasons, the cited art fails to render obvious independent claims 29 and 59. Claim 30, 39, 41, 46, 52, 59, and 61 each depend from claim 22 and are therefore allowable for at the same reasons.

With further regard to the rejection of claim 59, this claim requires "at least one water-soluble organic solvent at a concentration from about 10%$^{w/w}$ to about 40%$^{w/w}$ to selectively precipitate higher molecular weight RNA from the biological sample, and precipitating purified high molecular weight RNA from the biological sample." Neither Chomczynski nor Chen disclose or contemplate mixing at least one water-soluble organic solvent at this concentration range precipitating higher molecular weight RNA from the sample. As Examiner acknowledges, Chen discloses adding "an equal volume of isopropanol." Chen, page 6, Working Example 2, step (5) (Emphasis added). Thus, Chen clearly fails to disclose or contemplate adding at least one water-soluble organic solvent at a concentration from about 10%$^{w/w}$ to about 40%$^{w/w}$ or selectively precipitating higher molecular weight RNA from a biological sample. Chomczynski also fails to provide a disclosure that would remedy this deficiency.

Examiner also takes issue with the use of the term "selectively," stating "the word 'selectively' is not defined in such a manner so as to preclude the isolation of lower molecular weight RNA." Applicant submits that the claim does not require the preclusion of an incidental amount of lower molecular weight RNA. The term "selectively" in its normal everyday use is an adverb of the word "selective," which means "tending to select" or "highly specific activity or

effect." Merriam Webster's Collegiate Dictionary 10th Edition, Merriam-Webster, Inc., 1996, page 1059. Thus, the term as used in present claim 59 is simply meant to indicate that this method results in "highly specific effect" of precipitating higher molecular weight RNA. The cited art fails to disclose or contemplate any compositions or methods that would "selectively" precipitate higher molecular weight RNA. For at least these reasons, the cited art fails to render obvious claim 59 and Applicant respectfully requests that the rejection be withdrawn.

Examiner rejects claims 47-51 under 35 U.S.C. § 103(a) as allegedly unpatentable over Chen in view of Chomczynski, and further in view of Focus (1998) 20(2):36 ("Focus"). Applicant respectfully disagrees.

As discussed above, neither Chen nor Chomczynski individually or in combination discloses or contemplates compositions or methods that yield "RNA that does not reveal the presence of DNA when assayed by reverse transcription polymerase chain reaction (RT-PCR)," as required by claims 47-51. Focus, cited by Examiner as allegedly teaching "an intermediate centrifugation step," fails to provide a disclosure that would remedy this deficiency.

Examiner states that an "ordinary practitioner would have been motivated to perform this centrifugation since Focus notes that the centrifugation will 'pellet polysaccharides (also pellets genomic DNA)', so that the centrifugation step will enhance the purity and separation of the RNA from contaminating genomic DNA, as desired by Chen." Chen does not express a desire to yield RNA that is free of contamination of genomic DNA. In fact, Chen states "[t]he aim of the present invention is to provide a total RNA extractant, and method of producing same which enables the time for extraction of total RNA (including also rRNA, tRNA and mRNA) to be further shortened, is easily made, and has higher extraction efficiency; and a method of extracting total RNA by a simpler and quicker procedure." Chen, page 4, last

paragraph. Thus, Chen is not concerned with the purity of the sample of RNA. Moreover, as Applicant has clearly shown in the Declaration, Chen does not yield uncontaminated RNA, Chen yields RNA that is clearly contaminated with DNA as detected when the RNA is assayed by RT-PCR.

Finally, Focus does not teach a method that will yield RNA that does not reveal the presence of DNA when assayed by RT-PCR. Focus is concerned with methods of using the Trizol® reagent. In the response to the question "What can I do to avoid genomic DNA contamination of my RNA in RT-PCR applications?" (*see* Focus, page 36), Focus clearly states that when using the Trizol reagent, in order to get rid of contaminating genomic DNA, the RNA must be treated with "amplification grade DNase I." *Id.* Thus, the cited references individually or in combination fail to disclose or contemplate a method that will yield RNA that does not reveal the presence of DNA when assayed by RT-PCR.

For at least these reasons, the cited art fails to render obvious independent claims 47 and 48. Claim 49-51 each depend from at least one of claim 47 or 48 and are therefore allowable for at the same reasons.

**CONCLUSION**

As a result of the remarks given herein, Applicant submits that the rejections of the pending claims have been overcome. Therefore, Applicant respectfully submits that this case is in condition for allowance and requests allowance of the pending claims.

If Examiner believes any detailed language of the claims requires further discussion, Examiner is respectfully asked to telephone the undersigned attorney so that the matter may be promptly resolved. It is believed that no fee is due for this filing. If any fee is deemed due, consider this as an authorization to charge Deposit Account 23-3000 therefor.

Respectfully submitted,

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